Beyond Transplantation

Third Annual Samuel Jason Mixter Lecture

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It is an honor for me to present the Third Annual Samuel Jason Mixter Lecture before this distinguished audience. The subject of this report is the field of transplantation in its broadest definition: what has been achieved, and what may lie ahead. It is appropriate that this summary be presented in this forum, since many of the creative insights and innovations have come from New England surgeons. I will focus on a few of these contributions, but this in no way is intended to diminish the impact of the efforts of many others, both here in New England as well as in other centers in the United States and abroad.

In simple terms, surgeons treat patients by taking things out, putting things in, or moving things around. Organ transplantation is an extreme form of reconstructive surgery, and involves replacing lost function by putting something in. After years of careful experimental work, first in the laboratory and then in the clinical setting, surgeons and physicians at the Peter Bent Brigham Hospital, Boston, were poised to undertake organ transplantation in the mid-1950s. Joseph Murray, MD (Fig 1), your current president, headed the team that performed the first successful organ transplant in 1954.[1] It was a kidney, transplanted from an identical twin into his severely ill brother. The immunologic barrier of placing tissue from one nongenetically identical individual into another was overcome again at the Peter Bent Brigham Hospital under Dr Murray's direction in the early 1960s. These sentinel achievements marked a new era in medicine and surgery, as well as created new fields of scientific investigation such as transplantation biology and immunology. One of the very important lessons to learn from these events is that they were not isolated occurrences of good fortune, but rather the culmination of years of thoughtful research in the laboratory and an approach to treatment of patients in a new way because of dissatisfaction with current methods.

Renal transplantation and bone marrow transplantation evolved rapidly over the next 25 years, but the transplantation of other organs remained largely experimental until the late 1970s. With the advent of better immunosuppression, standardized surgical and anesthetic techniques, and the ability to monitor and control rejection, extrarenal organ transplantation underwent explosive growth. At the end of 1986, there were almost 100 heart transplantation centers, 41 liver transplantation centers, and 27 pancreatic transplant centers. Cumulatively, over 2000 liver transplants, over 3000 heart transplants, and almost 500 pancreas transplants had been performed by the end of 1986 in the United States (Fig 2). The Boston Center for Liver

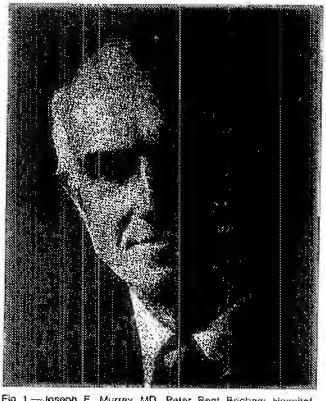

Fig 1.—Joseph E. Murray, MD, Peter Bent Brigham Hospital, Boston, surgeon who performed world's first successful organ transplant in 1954, that of a kidney into an identical twin.

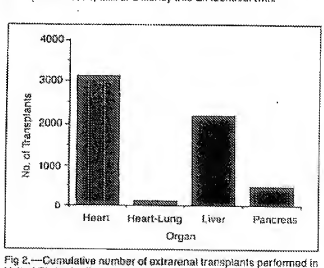

Fig 2.—Cumulative number of extrarenal transplants performed in United States by December 1986.

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Transplantation was created in January 1984 and was the sixth center to offer liver transplantation; now, as I mentioned, there are over 40 such centers.[2]

These achievements are truly remarkable, and the life that is returned to patients at times seems miraculous. However, the practice of transplantation is not without problems. I would like to share with you the experience of pediatric liver transplantation as we currently must practice it in the Boston area. The patient shown in Fig 3 was cared for by our program in 1986. This photograph was obtained while we continued to treat him as an outpatient. We make every effort to avoid hospitalization to prevent colonization by hospital-acquired resistant organisms and to keep the children with their families. We had been searching nationally for an appropriate donor organ for several months as the patient's condition progressively deteriorated. His course was complicated by marked malnutrition, portal hypertension, episodes of gastrointestinal tract bleeding, and systemic sepsis. Five days after transplantation, he died of overwhelming sepsis from a hospital-acquired organism. His condition was a direct result of the critical donor organ shortage among small children. Between 12% and 40% of infants will die before liver transplantation for lack of a donor, and many others will become so critically ill that the success of transplantation is diminished.[3] Because of the donor scarcity and the short preservation times currently allowable for a liver, teams must simultaneously harvest donor organs from as far away as 2000 miles. To accomplish this successfully, private jet aircraft are needed to shorten transportation time

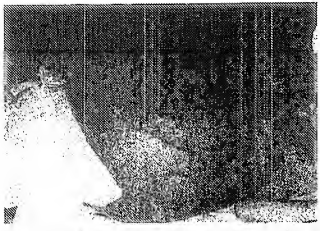


Fig 3.—Infant with marked cirrhosis from biliary atresia, leading to portal hypertension, malnutrition, gastrointestinal tract bleeding, and sepsis.

Fig 4.—Chartered aircraft for organ retrieval.

Fig 5.—Left, Paul S. Russell, MD, chief of transplantation at Massachusetts General Hospital, Boston. Right, John F. Burke, MD, chief of trauma services at Massachusetts General Hospital and creator of artificial dermis for massively burned patients.

(Fig 4). Often, we also employ helicopters to fly to the airport, to the donor hospital, and then back to the hospital to minimize preservation time. This need adds enormous cost and logistical complexity to the operative effort, thereby increasing the risk.

Small children require tiny livers, often from newborn donors. The vascular and biliary anastomoses are difficult, again increasing the risk of graft failure in the absence of artificial hepatic support. However, despite the many limitations, we all recognize that transplantation does work. It does save lives. The critical issue we must face is whether organ transplantation realistically can ever match the need. As an example, liver disease accounts for 30 000 deaths per year in the United States, with an estimated annual cost of $14 billion to the US health care system.[4] Ten million patients in the United States suffer from diabetes mellitus, of whom 600 000 are insulin dependent. Thirty percent of these patients have the devastating complications of diabetes. In 1984 dollars, their care resulted in a cost of $13.75 billion to the US economy.[5] In 1986, 924 liver transplants and 140 pancreatic transplants were performed in the United States. Even if the organ donor pool were to double or triple, it would fall far short of answering the need. Thus, major problems with organ transplantation as currently practiced include donor scarcity, expense, technical difficulty, and labor-intensive and complex care.

Surgeons have recognized these shortcomings for many years and have looked beyond transplantation for solutions. Paul Russell, MD (Fig 6, left), from our midst summarized new approaches to organ replacement when he wrote a review article on selective cell transplantation in 1985.[6] He clearly stated that if there was an effective way to transplant only those important functional cellular elements of an organ, there would be many conceptual advantages over organ transplantation. Researchers have tried for 15 years to solve the problem of islet cell transplantation, for example. Many others have worked on hepatocyte transplantation as a way to support patients with liver failure.[7] And so, cell transplantation has become a conceptual alternative, although now it is still highly experimental. In the 1970s, John Burke, MD (Fig 5, right), grappled with the problem of the massive burn wound. He realized that early excision and coverage was the cornerstone of suc-

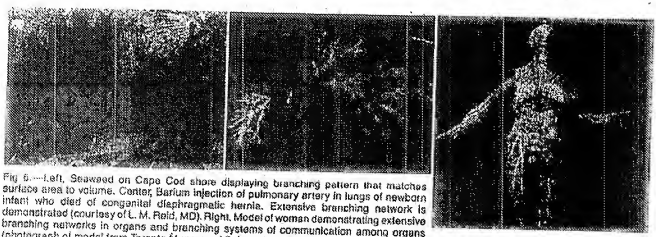
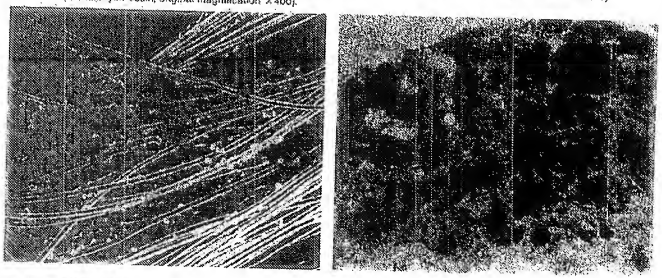


Fig 6.—Left, Seaweed on Cape Cod shore displaying branching pattern that matches surface area to volume. Center, Barium injection of pulmonary artery in lungs of newborn infant who died of congenital diaphragmatic hernia. Extensive branching network is demonstrated (courtesy of L. M. Reid, MD). Right, Model of woman demonstrating extensive branching networks in organs and branching systems of communication among organs (photograph of model from Toronto Museum of Science).

Fig 7.—Left, Scanning electron micrograph of polymer fibers with attached hepatocytes (original magnification ×121). Right, Implant of rat hepatocytes in omentum one week after implantation. Note active mitosis and vascularity (hematoxylin-eosin, original magnification ×400).



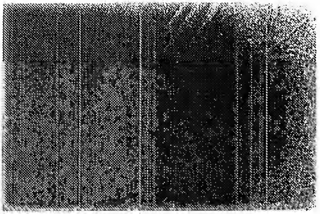


Fig 8.—Bovine aortic endothelial cells migrating into gel of complex biomatrix from polymer fibers (original magnification ×120).

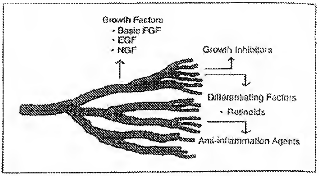


Fig 9.—Diagram demonstrating potential of slow release of biologically active macromolecules from polymer matrices. FGF indicates fibroblast growth factor; EGF, epidermal growth factor; and NGF, nerve growth factor.

cessful management. This approach, however, was limited by the availability of donor skin. Dr Burke began to conceptualize creating an artificial wound coverage, a matrix of neodermis that would be manufactured from natural elements. Working with Ioonas Yannas, MD, at Massachusetts Institute of Technology, Cambridge, Mass, an artificial dermis was designed from collagen and glycosaminoglycans. It was acellular and temporarily covered by a Silastic membrane. When placed onto an open burn wound, cellular migration and vascular ingrowth into the neodermis occurred, and the matrix was remodeled over time, becoming a living element. Epidermal elements from expanded skin grafts were gradually placed on the new dermis after vascular ingrowth occurred.[6] This concept and its successful use will clearly be one of the hallmark contributions to the management of patients in the latter half of the 20th century. Dr Burke and his colleagues were able to solve the problem of creating a new organ in two dimensions by using natural substances in a creative way.

We wondered whether we could apply these principles of cell transplantation and artificial matrix to three-dimensional systems in the design of visceral organs. What would be necessary? One would need dissociated parenchymal cells and an appropriate biodegradable scaffold that would allow the cells to remain viable by diffusion, promote vascular ingrowth, and permit cellular proliferation and function. The design of such a system is a major problem requiring expertise in cell and developmental biology, polymer technology, and biomedical engineering.

The rationale for such a proposal is based on several biologic observations. Every structure in living organisms undergoes constant renewal, remodeling, and replacement. Dissociated structural cells placed in cell culture tend toward reforming their structures. Their ability to do so depends on the conditions and cues provided in culture. Examples include endothelial cells that will form tubes in vitro and biliary cells that will form ducts in vitro.[8,9] Normal organ parenchymal cells are anchorage dependent. If dissociated cells are placed into mature tissue as a suspension without cell attachment, they may have a difficult time finding attachment sites that will allow for proper polarity and cell function. This may limit the total number of implanted cells that would remain viable to organize, proliferate, and function. Finally, but very important, tissue cannot be implanted in volumes of greater than 2 to 3 mm^3 because nutrition, gas exchange, and elimination of waste products is limited by this maximum diffusion distance.[11] We evolved the concept of the engineered creation of a new organ in situ by placing functional, dissociated cells onto biodegradable artificial polymers in culture and then placing this polymer-cell scaffold into a host where vascularization, growth, and function could occur. We have termed this process *chimeric neomorphogenesis*.[12]

The technique involves the harvest of the appropriate parenchymal cell type and placement into cell culture on polymer matrices. The ability then exists to manipulate those cells while in culture. For example, one could expand the number of cells in culture, offering the opportunity of using a small sample of the patient's own cells and allowing proliferation to achieve adequate numbers to replace lost function. One could insert missing genes for protein products such as factor VIII using the methods of genetic engineering. One might also manipulate antigenic surface determinants or eliminate unwanted antigen-presenting cells, thereby decreasing the likelihood of immune rejection. The cell-polymer scaffolds are then placed back into patients in appropriate locations under the proper conditions. The major components of this technique are (1) the use of biodegradable polymers; (2) cell viability supported temporarily by diffusion; (3) proliferation and organization of cells; (4) vascularization of the growing cell mass; and (5) proper cell function in the context of new structure. One must emphasize the importance of vascularization to allow cells to have adequate nutrient, gas, and waste exchange. The design of the polymer scaffold must allow all cells access to the environment until vascular ingrowth occurs.

This work has been done in close collaboration with Robert Langer, ScD, and his group at the Massachusetts Institute of Technology. The initial design was that of a small wafer of biodegradable polyanhydride. Cells were seeded in a monolayer onto the wafer in culture and then placed into a recipient animal while on the disk. Our initial experiments were not very successful. We thought this was most likely due to cell number and cell density that were inadequate for successful engraftment. In this context, we began to address the question of growth of multicellular organisms. How does Mother Nature solve the problem of three-dimensional growth? As a mass of cells enlarges, the surface area increases only as the square of the radius, but the volume increases as the cube of the radius. How does nature tackle this mismatch so that the cells on the interior can be nourished? Nature uses branching networks to achieve this goal of matching surface area to volume. She

uses it both in the animal and the plant worlds. All organs are composed of intertwined branching networks and all communication between organs is accomplished by branching systems. Indeed, animals are structurally not much different from plants in this fundamental repeating pattern. To be successful in their niche, animals must think, react, and move; hence, the developed nervous system, the covering of the skin, and the mobility allowed by a musculoskeletal system (Fig 6). But, in essence, we are branching networks, much as plants are.[12]

Several experiments have been performed to test the feasibility of this concept. Biodegradable polymers of fiber networks are constructed. Cell suspensions from the liver, intestine, or pancreas are placed on polymers and the polymer-cell scaffold is maintained in culture for four days, and then it is reimplanted in the animal. Sites tested have included the omentum, retroperitoneum, and subcutaneous tissue. Figure 7, left, is a scanning electron micrograph of dissociated hepatocytes that have been placed onto polymer fibers of polyglactin and cultured for several days. There are many healthy hepatocytes and others that appear to be degenerating. Figure 7, right, is a hepatocyte implant in the omentum one week after engraftment. It shows very viable and healthy hepatocytes, as well as mitotic figures indicating cell proliferation in the growing mass. Visible as well are blood vessels throughout the implant and evidence of bile canaliculi as specialized areas of the hepatocyte membrane. We have placed aortic endothelial cells onto polymer fibers and suspended them in gels of complex biomatrix (Fig 8). We have observed proliferation of the cells on the matrix and migration of the cells off of the fibers into the gel in an organized manner reminiscent of capillary branching.

The polymers we have employed are man-made and therefore allow great flexibility in composition, configuration, and control. We can engineer tensile strength, rate of degradation, and suitability for cell attachment. We may be able to modify the inflammatory response and cell attachment by modifying the material or by coating it. Growth factors, growth inhibitors, and differentiation factors can be incorporated directly into the polymer matrix (Fig 9). As the polymer erodes, these factors can be released in a biologically active form to stimulate the tissue, signaling proliferation or differentiation. The polymers can also be placed with cells attached into three-dimensional gels of collagen or complex biomatrices; they then may be studied with time-lapse video photography. This should allow the in vitro study of cell-cell interactions. We are now rigorously studying optimal conditions for cell attachment. By preconditioning polymer fibers with various buffers, the polymer surface is roughened, thereby increasing cell attachment. We have also coated polymer fibers with collagen, gelatin, and fibronectin to increase cell attachment.

The year 2000 marks not only the beginning of a new century but the dawn of a new millenium. Cell transplantation holds great promise in the treatment of many diseases. Much work needs to be done, but our hope is that someday cellular chimeras will provide replacement tissue for patients as an alternative to organ transplantation as currently practiced.

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